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Review

Pathogens of marine bivalves in Maine (USA): A historical perspective

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ABSTRACT

Shellfish aquaculture (in particular oyster cultivation) has the potential to play a significant role in refreshing the coastal economy in the state of Maine (USA). Although ocean warming and acidification are often listed as the primary manifestations of climate change in the marine environment, the issue of expanding geographic ranges of parasites and pathogens is an equally serious threat to shellfish populations. Protozoan parasites of the genera *Bonamia*, *Haplosporidium*, and *Perkinsus*, the bacterial pathogen responsible for Roseovarius oyster disease, and the disease condition is known as disseminated neoplasia are currently recognized as some of the significant threats to natural and farmed bivalve populations. We have analyzed the peer-reviewed literature for reports of these pathogens/conditions in Maine. Most reports focus on oysters from the Damariscotta River Estuary and are the result of directed studies into the biology of a particular pathogen rather than the result of intensive monitoring programs. The sampling effort could be interpreted in several ways including; the pathogens/conditions not impacting the annual harvest to any great extent due to limited distribution of the causative agent, the oysters developing a tolerance to the diseases, or just a lack of resources directed at studying this topic. With the shellfish aquaculture industry expected to grow in the next several decades, we recommend that a rigorous and sustained survey of parasitic diseases and believe that such an effort is fundamental to the success, resilience, and well-being of Maine's shellfish aquaculture industry.

1. Introduction

Over the past several decades, the fishery-based economy of coastal Maine has seen a decline in economic and ecological diversity as wild stocks of harvested fish have collapsed (e.g., cod) (Meng et al., 2016; Pershing et al., 2015). This decrease in fishery stocks has placed the industry in the vulnerable position of a monoculture economy, depending primarily on lobsters (Steneck et al., 2011). One critical component to revitalizing the coastal economy while preserving Maine's maritime culture is to complement the rebuilding of wild fish stocks with the development of a robust aquaculture industry. Shellfish aquaculture – of oysters in particular – has been increasing in recent years and has the potential to play a significant role in revitalizing Maine's coastal economy. In addition to being a valuable resource for coastal economies, oysters are considered ecosystem engineers; they influence estuarine water quality, provide protection and habitat for other species, and provide a significant food resource for humans (Dumbauld et al., 2009; Humphries et al., 2011; Rick et al., 2016). The historical record indicates that natural populations of bivalves were once an abundant source of food for the coastal inhabitants, however traditional local harvesting of the natural populations is being substituted worldwide by semi-intensive aquaculture operations (Larsen et al., 2013).

Given the rapid developments of coastal aquaculture in recent times, ensuring the survival of cultured bivalves to marketable sizes has become a top priority for producers, resource managers, and other stakeholders. Bivalve researchers have therefore focused their efforts on understanding the threats to shellfish populations due to biological agents such as parasitic protozoa and bacterial pathogens (Coen and Bishop, 2015; Dégremont et al., 2015; Shinn et al., 2014). The coast of

Abbreviations: DN, disseminated neoplasia; MSX, multinucleated sphere X; ROD, Roseovarius oyster disease

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Maine has long been viewed as an ideal location for bivalve aquaculture due in part to its relatively pristine environment, miles of shoreline, and historically low occurrence of shellfish die-offs due to disease-causing agents. However, recent studies (Marquis et al., 2015; Messerman and Bowden, 2016; Messerman et al., 2014; Miles, 2016) have suggested that there are many biological threats to shellfish in coastal Maine, and that risk levels may be increasing due to northward advances of previously undetected threats. Environmental risks often become the focus of study only after damage has been done; in coastal Maine, we have the opportunity to establish a baseline in the early phase of bivalve aquaculture growth. Herein, we focus on summarizing the state of knowledge regarding some of the major biological threats to shellfish populations along the coast of Maine, USA. These threats include those from the parasitic protozoan genera *Perkinsus*, *Haplosporidium*, *Marteilia*, and *Bonamia*, as well as from the bacterial pathogen Roseovarius oyster disease (ROD; formerly JOD, juvenile oyster disease), and the disease condition known as disseminated neoplasia (DN) (Barber, 2004; Boettcher et al., 2005). It must be recognized that global warming and ocean acidification are other key factors affecting the health and well-being of marine mollusks (Boulais et al., 2017; Dineshram et al., 2016; Goncalves et al., 2017; Goncalves et al., 2016; Wang et al., 2016). It is quite likely that these climate-driven changes to the marine environment will interact in complex ways with threats from the biological agents to exacerbate the risk to shellfish populations, especially in regions like the Gulf of Maine that are experiencing enhanced rates of change relative to other locations (Balch et al., 2012; Pershing et al., 2015). However, direct linkages between climate change and both the prevalence and spread of shellfish diseases remain unclear and will require major research efforts to understand. This review, in the context of programs like the Sustainable Ecological Aquaculture Network (SEANET; https://umaine.edu/epscor/seanet), should help to develop a better understanding of the challenges that are faced by the Maine shellfish industry to ensure its continued growth and sustainability. We also discuss future perspectives and our vision for shellfish aquaculture and related research in light of the current threats.

2. Materials and methods

The SCOPUS database (http://www.info.sciverse.com/scopus/) contains over 20,500 titles from 5000 publishers worldwide with > 60 million records, and it goes back to 1823. We searched for peer–reviewed articles published starting from 1950 until 2016 with titles or abstracts containing particular protozoan parasite taxonomic or disease name strings and references to Maine (Table 1) as reported elsewhere (Fernández Robledo et al., 2014; Ward and Lafferty, 2004); no records of meeting proceedings were included. References were imported to EndNote (Thompson-Reuters), and titles and abstracts were manually curated by eliminating duplicities, searched for each of the parasites/diseases and sorted by year, and the resulting number of publications was then used to build an Excel spreadsheet to generate the plots.

Table 1
Search strings for pathogens and bivalve groups.

Parasite/condition	Search string
Disseminated neoplasia	DN, Disseminated neoplasia
Bonamia/Bonamiosis	*Bonamia*, Bonamiosis, *Microcytos*
Haplosporidium/MSX	*Haplosporidium*, MSX
Perkinsus spp./Dermo	Dermo, *Dermocystidium*, Perkinsosis
Aliiroseovarius crassostreae/ROD	ROD, JOD, Roseovarius
Host	
Clam	Clam, *Macoma*, *Mya*
Mussel	Mussel, *Mytilus*
Oyster	Oyster, *Crassostrea*, *Ostrea*

3. Results and discussion

The number of papers in the literature (SCOPUS Database) reporting on the genera *Bonamia*, *Haplosporidium*, and *Perkinsus*, and the conditions disseminated neoplasia (DN) and Roseovarius oyster disease (ROD) were plotted over time (Fig. 1). Overall, the number of papers focusing on these major parasite genera and bivalve conditions was low; the highest and lowest number of papers corresponded to ROD (12 papers) and *Bonamia ostreae* (6 papers), respectively. Here we describe the conditions and pathogens in chronological order reported for the state of Maine.

3.1. Disseminated neoplasia (DN)

DN is a form of lethal cancer that affects soft shell clams (*Mya arenaria*) locally known as “steamers.” Heavily affected specimens are recognized by very dense hemolymph when withdrawn from the adductor muscle (Fig. 2A) and easily identified under the microscope by round cells that do not spread when deposited on a glass slide (Fig. 2B). The first reference to DN in *M. arenaria* from Maine corresponds to a survey of clams from Portland, Goose Cove, and Searsport in 1978 by Brown et al. (Brown et al., 1978). Only clams from Searsport were diseased with a prevalence of 19%. Since the first report, there have been eight references to DN in Maine, with one study focusing on discerning the molecular basis for naturally occurring diseases (Walker and Bottger, 2008) and another focused on the in vitro culture of the neoplastic cells (Walker et al., 2009). DN represents the first case of horizontal transmission of clonal cancer cells between individuals in marine bivalves and the third known case in the animal kingdom (Metzger et al., 2015) (Fig. 2C). The genotypes of neoplastic cells from clams sampled in locations on the East Coast of North America (New York and Maine in the USA, and Prince Edward Island in Canada) are nearly identical and differ from those of the host animal (Metzger et al., 2015). Similarly, analysis of DN in bivalves elsewhere indicates that neoplasias are attributable to independent transmissible cancer lineages and that clonal cancer cells can cross species boundaries (Metzger et al., 2016). Cancer cells in the golden carpet shell clam *Polititapes aureus* are all derived from the clam *Venerupis corrugata*, a different species living in the same geographical area. DN has been described in *C. virginica* from the Chesapeake Bay [reviewed in (Barber, 2004)]. Demonstrated horizontal transmission and the high prevalence of DN in *M. arenaria* from Maine (Nault, 2016), highlights the need for a close monitoring for this condition in *O. edulis* and *C. virginica*.

3.2. Bonamia ostreae

Bonamia ostreae (Fig. 3), a parasite of the European flat oyster *Ostrea edulis*, was described after mass mortalities of *O. edulis* in Brittany (France) in 1979 (Pichot et al., 1979). There is still active debate on where *B. ostreae* evolved and how it reached areas beyond its initial description area in France, but some data suggest that it may have originated from the West Coast of the U.S.A. from where *O. edulis* seed was transferred to France years previous to the detection of the disease there (Elston et al., 1986). Interestingly, some of the stocks where *B. ostreae* was detected in the West Coast were used in hatcheries in Maine (Elston et al., 1986). The first report of *B. ostreae* in Maine corresponds to *O. edulis* from sites in the Damariscotta River Estuary (DRE), where the parasite was identified in 1991–1992 with prevalence 34–45% respectively (Friedman and Perkins, 1994a). Oyster samples in the middle 1990's presented a lower prevalence (5%) (Zabaleta and Barber, 1996). The diagnosis was based on histological preparations, stained blood smears (Fig. 3A), and immunofluorescence (Friedman and Perkins, 1994b; Zabaleta and Barber, 1996). The transmission of the disease can occur between individuals (Elston et al., 1987), although the presence of an intermediate host has not been ruled out (Burreson and Ford, 2004; Lynch et al., 2007) and spores have been described for *Bonamia*

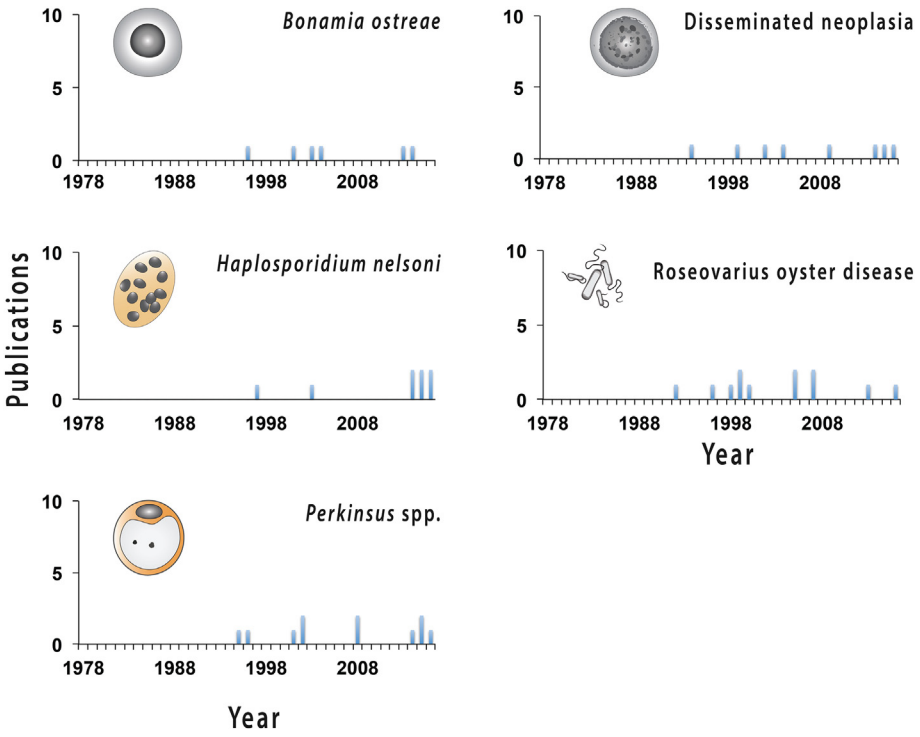

Fig. 1. Number of papers in the literature (SCOPUS Database) reporting in the genera *Bonamia*, *Haplosporidium*, and *Perkinsus*, and the conditions DN and ROD.

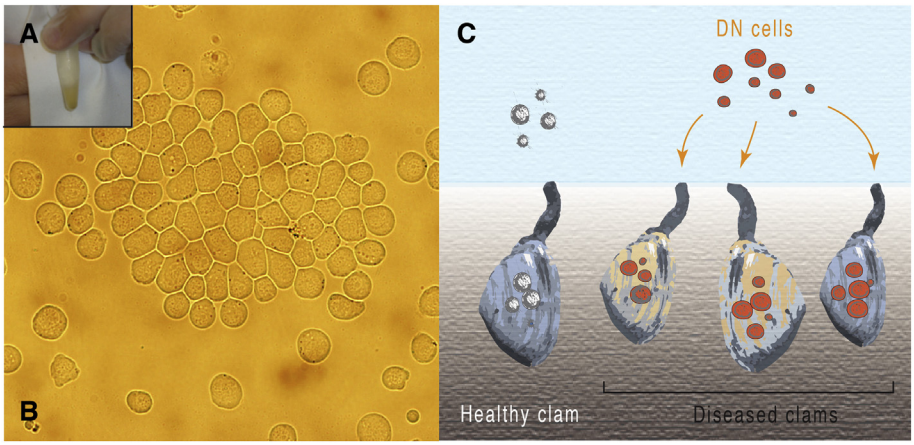

Fig. 2. Disseminated neoplasia (DN). A. Hemolymph withdrawn from clams with advanced DN is characterized by large number of cells giving the hemolymph a milky appearance. B. DN cells are rounded and have lost the ability to readily spread on to a glass surface and project pseudopodia. C. Transmission of DN occurs when diseased clams release into the environment DN cells, which are taken up by healthy clams.

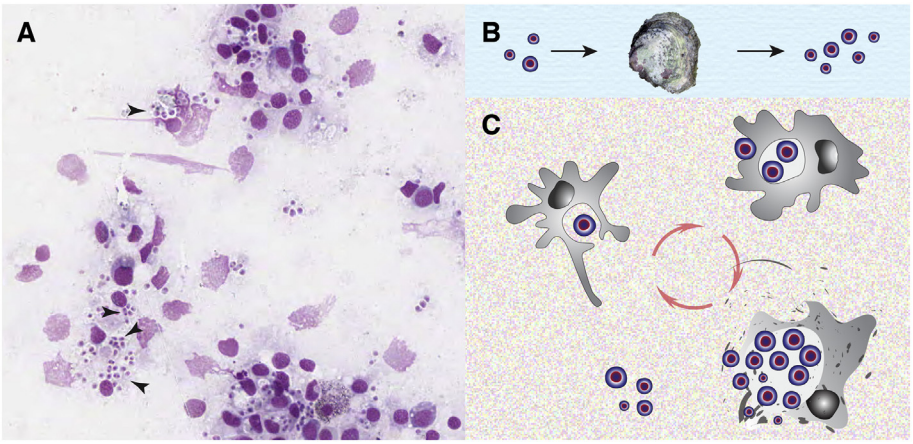

Fig. 3. *Bonamia ostreae*. A. Blood smear stained with hematoxylin and eosin. Note the groups of cells (arrowheads) inside the hemocytes. B. Transmission of *B. ostreae* remains unknown. Once inside the oyster, the parasite resides inside the hemocytes where it propagates, the parasite can also be found free-floating in the hemolymph after the rupture of the haemocytes.

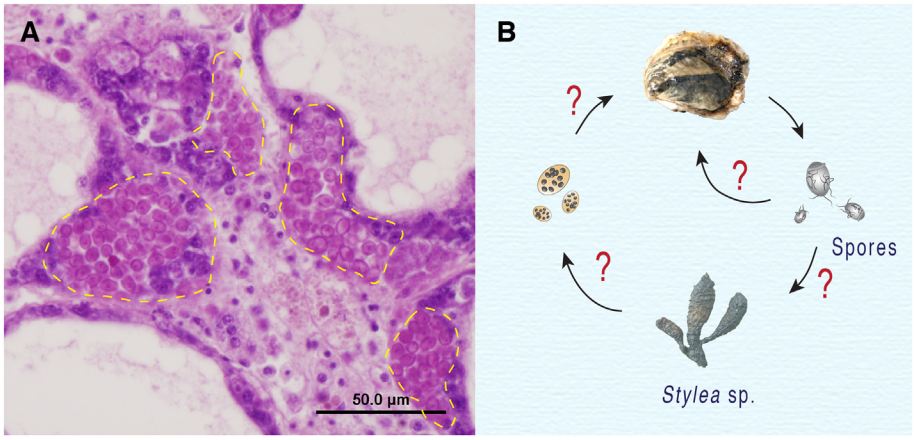

Fig. 4. *Haplosporidium nelsoni*. A. Digestive gland of *Crassostrea virginica* infected with *Haplosporidium nelsoni*; dash line indicates the presence of large number of *H. nelsoni* cells. B. Transmission of *H. nelsoni* is thought to be through spores. Recent studies have identified *H. nelsoni* DNA in the tunicate *Stylea* sp., but whether or not the tunicate is an intermediate host remains to be determined.

perspora (Carnegie et al., 2006). The parasite infects/resides inside the oyster hemocytes or extracellularly when the hemocytes dies and the parasites are released (Fig. 3A-3C). Altogether, there are only four references to *B. ostreae* in Maine with the latest reference dating back to 1998 with a prevalence below 1% of tested specimens (Carnegie and Barber, 2001). The highest prevalence reported corresponded to the DRE in 1992 (45%) (Fig. 1). There is a growing interest in bringing back the large-scale production of *O. edulis* to Maine (noting that there are still some wild populations derived from previous attempts to introduce it in Maine). "Extreme ice" winters appear to be responsible for preventing the spread of *B. ostreae* from imported flat oysters to native flat oyster populations in the Limfjord (Denmark) (Madsen et al., 2013). The extreme winters in Maine could favor the expansion of flat oyster aquaculture by keeping the incidence of *B. ostreae* low. However, as environmental conditions change in the Gulf of Maine, it is entirely possible that the prevalence of *B. ostreae* will also change.

3.3. *Haplosporidium nelsoni (multiple sphere X)*

Arguably, *H. nelsoni* (Fig. 4) is the most pertinent protozoan parasite to the oyster farmers and oyster gardeners in Maine. Two major die-offs of oysters in the 1990s and 2010 were blamed on this parasite, and reports of these events even reached local newspapers (Deese and Schmitt, 2011). Based on histology, the MSX parasite appears in the gills during in the early stages of infection (Burreson and Ford, 2004). The parasite is also observed in the digestive gland (Fig. 4A) and hemolymph (Burreson et al., 1988). The infective stage of *H. nelsoni* remains to be identified (Fig. 4B), but sporulation has been reported in spat (Barber et al., 1991) and transmission can occur between oysters separated by a 1 mm filter (Sunila et al., 2000). The first major epizootic event involving MSX was reported from the Piscataqua River Estuary (near the river mouth the river separates the states of New Hampshire and Maine) with prevalence ranging from 15% to 81% of sampled oysters. Unusually warm and dry weather conditions in the estuary appear to have favored the parasite (Barber et al., 1997). The report following the 2010 die-off indicated the presence of oysters positive for MSX (via PCR and histology) in the DRE, upper Sheepscot River, and Mill Cove in the New Meadows River. The previous survey included 15 sites between Casco Bay and Taunton Bay in Franklin, ME (Bouchard, 2012). Sites that were positive for MSX in 2010 were subsequently sampled in June/July 2011, but MSX was not detected at either of those locations (Bouchard, 2012). Given the absence of oyster die-offs since 2010 and the use of MSX-resistant oyster strains it is thought to have brought the threat of MSX is under control in Maine (Giray, 2016). Recent PCR-based studies have reported medium to high prevalence of *H. nelsoni* in farmed oysters in multiple areas in Maine, which supports a broader distribution of the etiological agent, whether this high prevalence would develop into disease and mortalities remains to be seen. The reported prevalence of MSX in oysters from the DRE during 2013–2014 varied between 26% and 60% (Marquis et al., 2015; Messerman and Bowden, 2016; Messerman et al., 2014; Miles, 2016) (MMS, SEK unpub. results) and in the 2017 summer we have recorded prevalence of MSX above 80% in DRE (JAFR, NDM, PDC, NRR, TJB, unpub. results). The latest survey expands the presence of MSX to the Bagaduce River, Basket Island, Maquoit Bay, Webhannet River with low prevalence (9–17%) across all sites (Marquis et al., 2015). These PCR-based findings indicate a range for *H. nelsoni* that is broader than earlier thought and increases the complexity for understanding the epizootiology of the disease. Switching from an MSX susceptible strain of oysters to an MSX resistant strain appears to have reduced the prevalence of the parasite when comparing surveys two years apart (2012–2014) (Dickey et al., 2017; Messerman et al., 2014). Management of oyster resources around MSX is quite challenging since the full *H. nelsoni* life cycle remains unknown; and at this point, we do not know if an intermediate host is required to complete the life cycle or how *H. nelsoni* enters the oyster (Arzul and Carnegie, 2015; Ford and Ashton-Alcox, 1998). In a survey for potential reservoir species for MSX in the DRE during the summer of 2012, up to 70% of the tunicate *Styela* sp. and about 30% of plankton sampled for were positive for MSX DNA using qPCR (Messerman and Bowden, 2016). Recently, we have also identified DNA sequences matching *H. nelsoni* in a survey from three sites in the DRE [J.A.F. et al., unpub. data]. These studies (Marquis et al., 2015; Messerman and Bowden, 2016; Messerman et al., 2014; Miles, 2016) reporting the prevalence of *H. nelsoni* are PCR based with no histopathological study associated nor do not have information on the genotype of the oysters sampled (diploid vs. triploid, oyster strains origin), which adds a layer of complexity to interpreting the prevalence in the absence of reported die-offs. Finally, the contribution of wild oyster populations to the parasite load in the water is unknown based on the above-mentioned gaps in the life cycle but also because any die-offs affecting wild oyster populations are likely to pass unnoticed, and most mollusk pathogens are not associated with gross signs of disease (Carnegie et al., 2016).

3.4. *Perkinsus spp. (Dermo)*

Perkinsus marinus is a protozoan parasite that has been studied in more detail than most other oyster parasites (Fernández Robledo et al., 2014). Trophozoites of the parasite can be easily distinguished in histology samples by their characteristic "signet ring" cell shape (Fig. 5A); with a direct life cycle, most of the life cycle stages have been characterized (Fig. 5B). *Perkinsus marinus* was first reported in Maine in the DRE; the protozoan was identified in *C. virginica* hemolymph using Ray's Fluid Thioglycollate Medium (RFTM) (Kleinschuster and Parent, 1995) (Fig. 1). At the time, a positive diagnostic by RFTM was associated to *P. marinus*, however it is now known that in the absence of

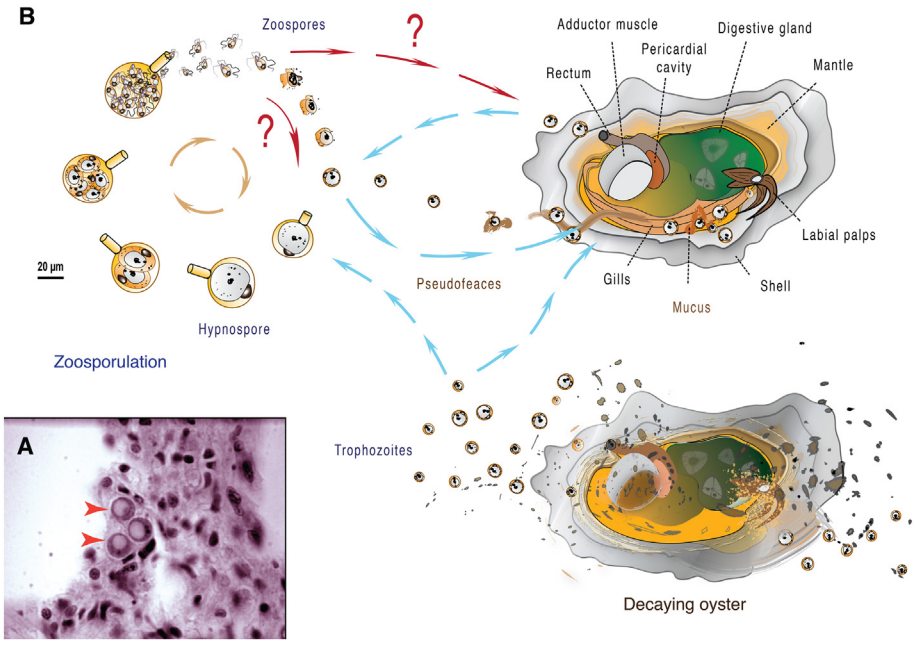

Fig. 5. *Perkinsus marinus*. A. Trophozoites (arrowheads) reside both intracellularly inside the hemocytes and extracellularly. B. Trophozoites in the water column are drawn in the oyster during filter-feeding. Although in some cases they may be released into the water with the pseudofeces, most of them are recognized and phagocytized by the hemocytes. Inside the phagosome-like vesicles trophozoites propagate and, eventually, the hemocyte disintegrates the released trophozoites that can be phagocytozed by neighboring hemocytes.

molecular assays the RFTM assay alone does not have the ability to differentiate *P. marinus* from *P. chesapeaki* (Berthe et al., 2004; Coss et al., 2001a; Coss et al., 2001b; Robledo et al., 1998) and indeed the same specimen can be co-infected by both host species (Arzul et al., 2012; Coss et al., 2001a; Coss et al., 2001b; Marquis et al., 2015; McLaughlin and Faisal, 1998; Reece et al., 2008). Since the first report of *Perkinsus*, there have been 11 references to *Perkinsus* spp. in Maine. In the 1990's the prevalence of *P. marinus* was qualified as "sub-clinical" (Kleinschuster and Parent, 1995); in the 2000's, the prevalence` for both *P. marinus* and *P. chesapeaki* was still low (Pecher et al., 2008). However, since the 2000's survey, we have reported using PCR-based assays a 15–65-fold increase of the *P. marinus* and *P. chesapeaki* prevalence over a period of 12 years in the DRE (Marquis et al., 2015). In the same study, we reported that an oyster positive for both *Perkinsus* spp. is 3.2 times as likely to be positive for a non-*Perkinsus* protozoan parasite. Early studies indicate that *P. chesapeaki* has a preference for infecting clams (Arzul et al., 2012; McLaughlin and Faisal, 1998; Reece et al., 2008); however, the latest survey in Maine shows an increase in the PCR-based prevalence of *P. chesapeaki* in oysters (Marquis et al., 2015). The PCR-based diagnostic was run on DNA obtained from samples including oyster's rectum, gill, and mantle. Hence, it would have included trophozoites inside hemocytes circulating in the tissues, trophozoites propagating extracellularly, and trophozoites on the surface of those organs. Like any other intracellular protozoan, the adherence to host tissues is part of the infection cycle (Singh et al., 2016; von Itzstein et al., 2008). In the case of *Perkinsus* spp., this adherence may even be part of the strategy of infection since trophozoites lack of mechanisms for active entry into host cells (e.g., gliding, apical complex). Indeed, it has been shown that the galectin (CvGal1) mediates the entry in the hemocytes (Tasumi and Vasta, 2007; Vasta et al., 2015), which can be found in the pallial cavity (Lau et al., 2018). Supporting the point that the oyster is infected once trophozoites reaches the pallial cavity is that the parasite is already actively expressing putative virulence genes in response to the contact with mucus (Pales Espinosa et al., 2014), translocation throughout the host body via the host's circulatory system occurs 12 days post exposure to zoospores (Wang et al., 2018), and *P. marinus* does not survive > 3 days in the water outside the host (Chu and Lund, 2006). Recently, the presence of hemocytes associated with mucus covering the pallial organs (mantle, gills, and body wall) have been implicated on the pathogenesis of *P. marinus* (Lau et al., 2018). Finally, all the *Perkinsus* spp. described so far are parasites of mollusks; the Perkinsozoa group split from the common ancestor circa 391 Ma ago (Okamoto and McFadden, 2008); they have evolved to adhere, enter the oyster, and survive inside the defense cells of mollusks (Vasta et al., 2015). Trophozoites reside both within the oyster hemocytes, which are responsible for immune defense and transport among other functions (Comesana et al., 2012; Morga et al., 2012; Morga et al., 2011) and extracellularly exposing the tissues other than blood to the activity of secreted proteases (Casas et al., 2008; Garreis et al., 1996a, 1996b; La Peyre et al., 1995; Ottinger et al., 2001). In the absence of histopathology studies that would also help to define of the extend of the damage and reaction of the hosts, hypothesis to explain these findings include new strains of *P. chesapeaki* adapting to host/environmental conditions in Maine, an increase in the aquaculture activities with more host getting infected and releasing trophozoites, and overall warmer temperatures as well (Saba et al., 2016). So far, no oyster die-offs have been ascribed to *Perkinsus* spp. in Maine even with the high PCR-based prevalence of the parasite. The lack of reported oyster mortalities in Maine due to *Perkinsus* may derive from oysters reaching the commercial size and being harvested before visible signs and mortalities; a situation already described in flat oysters infected with *B. ostreae* (Cáceres-Martínez et al., 1995). *Perkinsus* spp. thrives at higher temperature and salinity (Chu et al., 1993; Ford and Smolowitz, 2007; Queiroga et al., 2016); in vitro studies indicate that incubation at 4 °C of *Perkinsus* spp. results in decreased viability with limited metabolic activity and no proliferation, but only partially explains reduced parasite infection intensities in the colder months of the year (La Peyre et al., 2008). Most models for climate change predict an increase in the runoff from terrestrial ecosystems in the estuaries, linked to higher variance between wet and dry conditions (Balch et al., 2012), and an increase in the temperature in the Gulf of Maine (Saba et al., 2016). As an example, a buoy deployed in Penobscot Bay (www.neracoos.org) since 2001 has shown a salinity range of 17–35 psu. Taking a coastal buoy deployment in Penobscot Bay as a temperature baseline, there is currently < 1 day per year with a mean temperature over the critical Dermo threshold of 20 °C, whereas under 2 °C of warming, this increases to ~6 days per year, and under 4 °C of warming, to ~20 days per year. With the high prevalence of *P. marinus* in Maine oysters, unusually warm and dry weather conditions, a situation also associated to the die-off attributed to MSX (Barber et al., 1997), might result in a perfect storm positioned to hit the nascent oyster aquaculture industry.

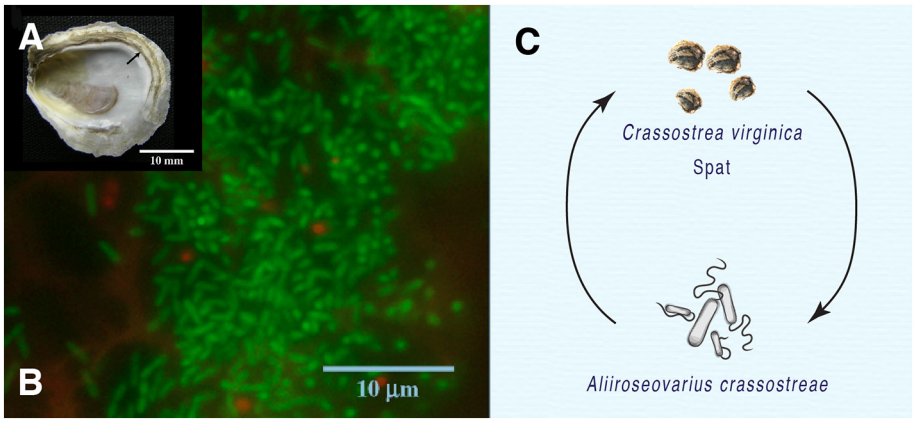

Fig. 6. *Aliiroseovarius crassostreae*. A. *Crassostrea virginica* affected with ROD. B. Inner shell from *Crassostrea virginica* containing bacteria stained with nucleic acid viability dye (Boardman et al., 2008). C. *Aliiroseovarius* spp. are components of the estuarine microbiome. *Aliiroseovarius crassostreae* affects mostly *C. virginica* spat causing heavy mortalities. (Panels A and B reproduced with permission from Elsevier).

3.5. Roseovarius oyster disease (ROD)

Caused by the α-proteobacterium *Aliiroseovarius crassostreae* (Maloy et al., 2007a; Maloy et al., 2007b), ROD can be tracked to 1988 (Boettcher et al., 1999; Bricelj et al., 1992) (Fig. 6). The bacterium was recovered from ROD-affected animals in numerous Northeast states in 1997 (Maloy et al., 2007a; Maloy et al., 2007b). Altogether there have been 14 papers mentioning ROD in Maine including PCR diagnostic development (Maloy et al., 2005), epizootiological studies (Maloy et al., 2007a; Maloy et al., 2007b), experimental challenges and survival (Dégremont, 2013; Gomez-Leon et al., 2008), and localization on juvenile oyster tissues (Boardman et al., 2008). Recently, in a study of the performance of selectively bred lines of eastern oyster, the available oyster strains performed very poorly in Maine waters with accumulated mortalities of 50% within three months of the deployment and reaching accumulated mortalities of 70% 12 months later (Proestou et al., 2016). The study mentioned that the mortality was coincident with increased ROD prevalence in that time of the year although samples were not examined for MSX, Dermo, or ROD (Proestou et al., 2016) making it more difficult to assign the mortality to any of those pathogens.

4. Future perspectives

Maine's growing aquaculture industry is attracting capital from private equity investors. Recently, FocusMaine, a non-profit business operating under the umbrella of the Maine State Chamber of Commerce, identified aquaculture as one of the sectors in Maine with high potential to create thousands of jobs over the next decade. However, the Gulf of Maine is a rapidly changing system, with some of the fastest rates of temperature change on the planet (Pershing et al., 2015), major declines in primary productivity, and increases in dissolved organic material entering from its adjacent watersheds (Huntington et al., 2016). These changes are rapidly altering the ecosystem, with consequences looming for fisheries (Katherine, 2013), biodiversity (Record et al., 2016) and key native species (Neckles, 2015). Managing the growth of the aquaculture industry in this rapidly changing system will be a challenge, and careful monitoring of pathogens will be critical. Expanding to the marine field (Record, 2017) “People's science” using highly-portable technology to perform DNA-based identification (Marx, 2015) can contribute to the growing knowledge base of the distribution and diversity of microbial pathogens in the marine and complement other well establish techniques (e.g., histopathology) that inform about the presence of the pathogen and the health status of the oysters.

We previously addressed the analysis of the peer-reviewed literature on protozoan parasites of bivalve mollusks (Fernández Robledo et al., 2014). Here, the limited number of papers precluded us from identifying trends in the body of published work other than providing a side–by– side comparison of the publication record for threats affecting mollusks in Maine. The low number of reports may be explained by the limited size of the shellfish aquaculture compared to the lobster industry in both landings and economic impact in the state and being low on the priority list compared to surveys for fecal contamination or harmful algal blooms. Monitoring the prevalence of pathogens and limiting the movement of stocks between water bodies has been the leading strategy for intervention. Increased monitoring effort has resulted in reports of the pathogens in new areas (Marquis et al., 2015); however, no reports of abnormal die-offs is a reminder the complexity for understanding the epizootiology of the diseases. In the cases of oyster aquaculture relying on seed from hatcheries, another intervention strategy is to use strains selected for resistance/tolerance to protozoan parasites, with the handicap that strain performance varies significantly across sites (Frank-Lawale et al., 2014; Proestou et al., 2016). A second approach involving selective breeding for oysters have resulted in dozens of families with 3.4 traits selected on average (Gjedrem et al., 2012). In general, it seems that breeding for higher resistance to one disease does not appear to confer greater resistance or susceptibility to another disease (Dégremont et al., 2015). In light of these challenges, a consortium of researchers is in the process of sequencing, assembling, and annotating the first reference genome for the eastern oyster (Gómez-Chiarri et al., 2015a; Gómez-Chiarri et al., 2015b) to accelerate and determine the genetic basis for resistance to diseases or other highly desired traits for aquaculture. With this initiative underway, it would be of great utility to develop an oyster genetic system (OGS) for interrogating the genome for identifying genes involved in different aspects of the oyster's biology (e.g., immune defense, shell formation, adaptations to global warming and ocean acidification) and highly desirable commercial traits (Fig. 7). Confirmation of the role of particular candidate genes in determining a particular trait will allow for more targeted and faster selection of broodstocks to improve overall breeding efforts.

The development of a genetic research system for commercially important species of shellfish has been pursued for > 30 years with mixed results. Gene delivery approaches that have been used in an attempt to introduce desirable traits into shellfish have included electroporation, lipofection, and microinjection (Boulo et al., 1996; Buchanan et al., 2001; Cadoret et al., 1997) but experiments were often short-lived and not maintained in subsequent generations. Genetic systems and the capabilities they enable (e.g., knock out specific genes) have been essential for assigning functions to new genes and describing new physiological processes, aspects especially relevant in less studied organisms compared to mammals and medical model systems. Also, recent developments in techniques for gene editing (e.g., Clustered regularly interspaced short palindromic repeats, CRISPR/Cas9) have revolutionized the way researchers interrogate genomes for gene function and to identify desirable genetic traits (Hille and Charpentier, 2016; Mojica and Montoliu, 2016). These approaches are quickly being adopted in fish and mollusk aquaculture research (Chakrapani et al.,

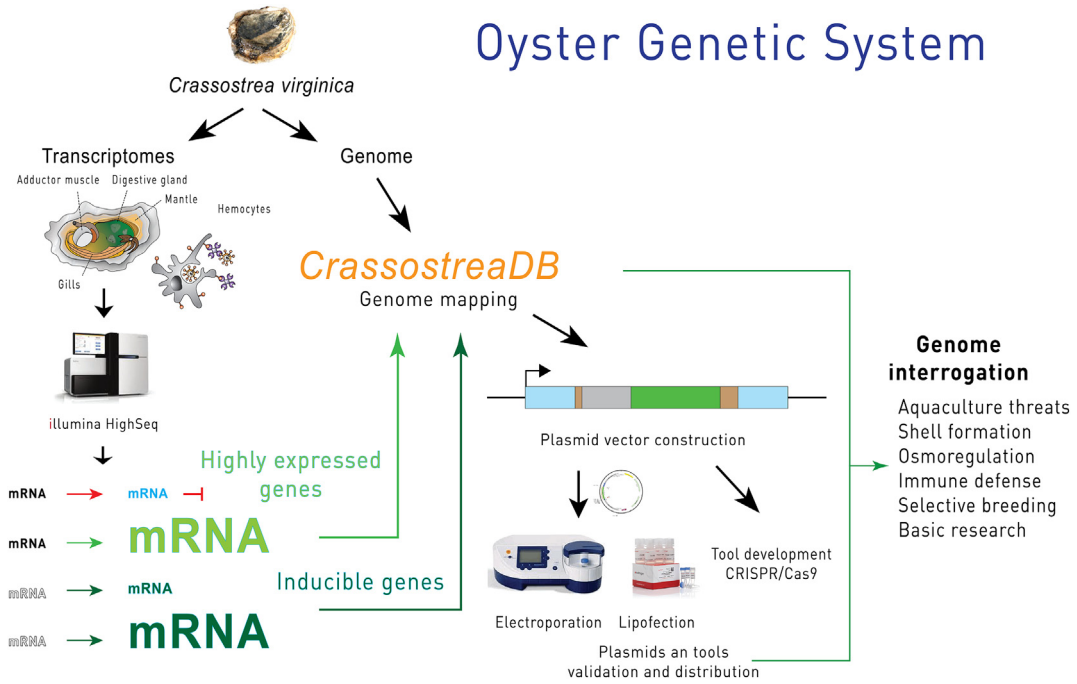

Fig. 7. Flow chart for developing the Oyster Genetic System. Transcriptome studies will be used to identify both highly expressed constitutive and inducible genes. These genes would be mapped to the genome to identify the promoters driving their transcription. Plasmid vectors based on these genes would be tested on oyster oocytes/zygotes and primary cell cultures using transfection by electroporation and lipofection. Once the plasmid and transfection conditions are optimized, other tools (e.g., Clustered regularly interspaced short palindromic repeats, CRISPR/Cas9) will be also developed. The oyster genetic system will be used for interrogating the oyster genome.

2016; Perry and Henry, 2015; Zhong et al., 2016). Moving the basic research to applied aquaculture is a step that is taking place slowly and under rigorous evaluation not without a high degree of controversy (Clausen and Longo, 2012; Tonelli et al., 2017; Ye et al., 2015).

Our review is a close-up historical snapshot of the record relating to protozoan parasites and bivalve diseases in bivalves from Maine. With more and more seafood reaching the table coming from aquaculture (FAO, 2012), it is important to understand pathogen baselines to maintain biosecurity at state, national, and international levels (Carnegie et al., 2016). It is also crucial to understand the parasite's biology, the interaction with the host, and the response to changing environmental conditions to have well-informed approaches for risk management. A baseline of waterborne parasites in commercial shellfisheries and aquaculture species is necessary to ensure responsible harvesting and production. This knowledge is particularly necessary to resolve any significant die-off of commercial shellfish species in the future and to contribute to the development of a sustainable and safe aquaculture.

Conflict of interest

We declare no conflicts of interest.

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References

Arzul, I., Carnegie, R.B., 2015. New perspective on the haplosporidian parasites of molluscs. J. Invertebr. Pathol. 131, 32–42.

Arzul, I., Chollet, B., Michel, J., Robert, M., Garcia, C., Joly, J.P., François, C., Miossec, L., 2012. One *Perkinsus* species may hide another: characterization of *Perkinsus* species present in clam production areas of France. Parasitology 139, 1757–1771.

Balch, W.M., Drapeau, D.T., Bowler, B.C., Huntington, T.G., 2012. Step-changes in the physical, chemical and biological characteristics of the Gulf of Maine, as documented by the GNATS time series. Mar. Ecol. Prog. Ser. 450, 11–35.

Barber, B.J., 2004. Neoplastic diseases of commercially important marine bivalves. Aquat. Living Resour. 17, 449–466.

Barber, R.D., Kanaley, S.A., Ford, S.E., 1991. Evidence for regular sporulation by *Haplosporidium nelsoni* (MSX) (Ascetospora; Haplosporidiidae) in spat of the American oyster, *Crassostrea virginica*. J Protozool 38, 305–306.

Barber, B.J., Langan, R., Howell, T.L., 1997. *Haplosporidium nelsoni* (MSX) epizootic in the Piscataqua River Estuary (Maine/New Hampshire, U.S.A.). J. Parasitol. 83, 148–150.

Berthe, F.C.J., Choi, K.S., Figueras, A., Soudant, P., Villalba, A., 2004. Perkinosis in Europe: current issues and research needs. Bull. Eur. Assoc. Fish Pathol. 24, 52–53.

Boardman, C.L., Maloy, A.P., Boettcher, K.J., 2008. Localization of the bacterial agent of juvenile oyster disease (*Roseovarius crassostreae*) within affected eastern oysters (*Crassostrea virginica*). J. Invertebr. Pathol. 97, 150–158.

Boettcher, K.J., Barber, B.J., Singer, J.T., 1999. Use of antibacterial agents to elucidate the etiology of juvenile oyster disease (JOD) in *Crassostrea virginica* and numerical dominance of an α- proteobacterium in JOD-affected animals. Appl. Environ. Microbiol. 65, 2534–2539.

Boettcher, K.J., Geaghan, K.K., Maloy, A.P., Barber, B.J., 2005. *Roseovarius crassostreae* sp. nov., a member of the Roseobacter clade and the apparent cause of juvenile oyster disease (JOD) in cultured eastern oysters. Int. J. Syst. Evol. Microbiol. 55, 1531–1537.

Bouchard, D., 2012. Response to an emerging shellfish pathogen: MSX in Maine oysters (*Crassostrea virginica*). 1VEC0PV MEU-G-12-002, pp. 1–2.

Boulais, M., Chenevert, K.J., Demey, A.T., Darrow, E.S., Robison, M.R., Roberts, J.P., Volety, A., 2017. Oyster reproduction is compromised by acidification experienced seasonally in coastal regions. Sci. Rep. 7, 13276.

Boulo, V., Cadoret, J.P., Le Marrec, F., Dorange, G., Miahle, E., 1996. Transient expression of luciferase reporter gene after lipofection in oyster (*Crassostrea gigas*) primary cell cultures. Mol. Mar. Biol. Biotechnol. 5, 167–174.

Bricelj, V.M., Ford, S.E., Borrero, F.J., Perkins, F.O., Rivara, G., Hillman, R.E., Elston, R.A., Chang, J., 1992. Unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica* (Gmelin). J. Shellfish Res. 11, 331–347.

Brown, R.S., Wolke, R.E., Saila, S.B., Brown, C.W., 1978. Prevalence of neoplasia in 10 New England populations of the soft-shell clam (*Mya arenaria*). Ann. N. Y. Acad. Sci. 298, 522–534.

Buchanan, J.T., Nickens, A.D., Cooper, R.K., Tiersch, T.R., 2001. Transfection of eastern

oyster (*Crassostrea virginica*) embryos. Mar. Biotechnol. (NY) 3, 322–335.
Burreson, E.M., Ford, S.E., 2004. A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease). Aquatic Liv Res 17, 499–517.
Burreson, E.M., Robinson, M.E., Villalba, A., 1988. A comparison of paraffin histology and hemolymph analysis for the diagnosis of *Haplosporidium nelsoni* (MSX) in *Crassostrea virginica* (Gmelin). J. Shellfish Res. 7, 19–23.
Cáceres-Martínez, J., Robledo, J.A.F., Figueras, A., 1995. Presence of *Bonamia* and its relation to age, growth rates and gonadal development of the flat oyster, *Ostrea edulis*, in the Ría de Vigo, Galicia (NW Spain). Aquaculture 130, 15–23.
Cadoret, J.P., Gendreau, S., Delecheneau, J.M., Rousseau, C., Mialhe, E., 1997. Microinjection of bivalve eggs: application in genetics. Mol. Mar. Biol. Biotechnol. 6, 72–77.
Carnegie, R.B., Barber, B.J., 2001. Growth and mortality of *Ostrea edulis* at two sites on the Damariscotta river estuary, Maine, USA. J. World Aquac. Soc. 32, 221–227.
Carnegie, R.B., Burreson, E.M., Hine, P.M., Stokes, N.A., Audemard, C., Bishop, M.J., Peterson, C.H., 2006. *Bonamia perspora* n. Sp. (Haplosporidia), a parasite of the oyster *Ostreola equestris*, is the first *Bonamia* species known to produce spores. J. Eukaryot. Microbiol. 53, 232–245.
Carnegie, R.B., Arzul, I., Bushek, D., 2016. Managing marine mollusc diseases in the context of regional and international commerce: policy issues and emerging concerns. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 371.
Casas, S.M., Reece, K.S., Li, Y., Moss, J.A., Villalba, A., La Peyre, J.F., 2008. Continuous culture of *Perkinsus mediterraneus*, a parasite of the European flat oyster *Ostrea edulis*, and characterization of its morphology, propagation, and extracellular proteins *in vitro*. J. Eukaryot. Microbiol. 55, 34–43.
Chakrapani, V., Patra, S.K., Panda, R.P., Rasal, K.D., Jayasankar, P., Barman, H.K., 2016. Establishing targeted carp TLR22 gene disruption via homologous recombination using CRISPR/Cas9. Dev. Comp. Immunol. 61, 242–247.
Chu, F.L., Lund, E.D., 2006. Viability, infectivity and fatty acid synthetic activity of *Perkinsus marinus* meront cells incubated in estuarine and artificial seawater. Dis. Aquat. Org. 71, 131–139.
Chu, F.L., Burreson, C.S., Volety, A., Constantin, G., 1993. *Perkinsus marinus* susceptibility in eastern (*Crassostrea virginica*) and Pacific (*Crassostrea gigas*) oysters: temperature and salinity effects. J. Shellfish Res. 12.
Clausen, R., Longo, S.B., 2012. The tragedy of the commodity and the farce of AquAdvantage Salmon. Dev Change 43, 229–251.
Coen, L.D., Bishop, M.J., 2015. The ecology, evolution, impacts and management of host-parasite interactions of marine molluscs. J. Invertebr. Pathol. 131, 177–211.
Comesana, P., Casas, S.M., Cao, A., Abollo, E., Arzul, I., Morga, B., Villalba, A., 2012. Comparison of haemocytic parameters among flat oyster *Ostrea edulis* stocks with different susceptibility to bonamiosis and the Pacific oyster *Crassostrea gigas*. J. Invertebr. Pathol. 109, 274–286.
Coss, C.A., Robledo, J.A.F., Vasta, G.R., 2001a. Fine structure of clonally propagated *in vitro* life stages of a *Perkinsus* sp. isolated from the Baltic clam *Macoma balthica*. J. Eukaryot. Microbiol. 48, 38–51.
Coss, C.A., Robledo, J.A.F., Ruiz, G.M., Vasta, G.R., 2001b. Description of *perkinsus andrewsi* n. sp. isolated from the Baltic clam (*Macoma balthica*) by characterization of the ribosomal RNA locus, and development of a species-specific PCR-based diagnostic assay. J. Eukaryot. Microbiol. 48, 52–61.
Deese, H., Schmitt, C., 2011. MSX Strikes Maine Oysters. The Working Waterfront.
Dégremont, L., 2013. Size and genotype affect resistance to mortality caused by OsHV-1 in *Crassostrea gigas*. Aquaculture 416–417, 129–134.
Dégremont, L., Garcia, C., Allen, S.K.Jr., 2015. Genetic improvement for disease resistance in oysters: a review. J. Invertebr. Pathol. 131, 226–241.
Dickey, D., Messerman, N.A., Bowden, T.J., 2017. Prevalence of the protozoan parasite *Haplosporidium nelsoni* in the eastern oyster, *Crassostrea virginica*, within the Damariscotta River Estuary, in Maine, USA, in 2014 and 2016 as measured by PCR. Bull. Eur. Ass. Fish Pathol. 37, 235–243 (In Press).
Dineshram, R., Chandramouli, K., Ko, G.W., Zhang, H., Qian, P.Y., Ravasi, T., Thiyagarajan, V., 2016. Quantitative analysis of oyster larval proteome provides new insights into the effects of multiple climate change stressors. Glob. Chang. Biol. 22, 2054–2068.
Dumbauld, B.R., Ruesink, J.L., Rumrill, S.S., 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in West Coast (USA) Estuaries. Aquaculture 290, 196–223.
Elston, R.A., Farley, C.A., Kent, M.L., 1986. Occurrence and significance of bonamiasis in European flat oysters *Ostrea edulis* in North America. Dis. Aquat. Org. 2, 49–54.
Elston, R.A., Kent, M.L., Wilkinson, M.T., 1987. Resistance of *Ostrea edulis* to *Bonamia ostreae* infection. Aquaculture 64, 237–242.
FAO, 2012. 2014 FAO yearbook. In: Fishery and aquaculture statistics. FAO, Rome, Italy, pp. 76.
Fernández Robledo, J.A., Vasta, G.R., Record, N.R., 2014. Protozoan parasites of bivalve molluscs: literature follows culture. PLoS One 9, e100872.
Ford, S.E., Ashton-Alcox, K.A., 1998. MSX - a review and update. J. Shellfish Res. 17.
Ford, S.E., Smolowitz, R., 2007. Infection dynamics of an oyster parasite in its newly expanded range. Mar. Biol. 151, 119–133.
Frank-Lawale, A., Allen, S.K., Dégremont, L., 2014. Breeding and domestication of eastern oyster (*Crassostrea virginica*) lines for culture in the Mid-Atlantic, USA: line development and mass selection for disease resistance. J. Shellfish Res. 33, 153–165.
Friedman, C.S., Perkins, F.O., 1994a. Range extension of *Bonamia ostreae* to Maine, U.S.A. J. Invertebr. Pathol. 64, 179–181.
Friedman, C.S., Perkins, F.O., 1994b. Presence of *Bonamia ostreae* among populations of the European flat oyster, *Ostrea edulis* Linne, in California, USA. J. Shellfish Res. 8, 133–137.
Garreis, K.A., La Peyre, J.F., Faisal, M., 1996a. The effects of *Perkinsus marinus* extracellular products and purified proteases on oyster defence parameters *in vitro*. Fish & Shellfish Immunol. 6, 581–597.
Garreis, K.A., La Peyre, J.F., Faisal, M., 1996b. The effects of *Perkinsus marinus* extracellular products and purified proteases on oyster defence parameters *in vitro*. Fish Shellfish Immun 6, 581–597.
Giray, C., 2016. Bivalve Shellfish Physiology, Pathogens, Diseases. Shellfish Biosecurity: Risks, Management, and Best Practices. University of Maine Hutchinson Center, Belfast.
Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. Aquaculture 350–353, 117–129.
Gómez-Chiarri, M., Warren, W.C., Guo, X., Proestou, D., 2015a. Developing tools for the study of molluscan immunity: the sequencing of the genome of the eastern oyster, *Crassostrea virginica*. Fish Shellfish Immunol. 46, 2–4.
Gómez-Chiarri, M., Guo, X., Tanguy, A., He, Y., Proestou, D., 2015b. The use of -omic tools in the study of disease processes in marine bivalve mollusks. J. Invertebr. Pathol. 131, 137–154.
Gomez-Leon, J., Villamill, L., Salger, S.A., Sallum, R.H., Remacha-Trivino, A., Leavitt, D.F., Gomez-Chiarri, M., 2008. Survival of eastern oysters *Crassostrea virginica* from three lines following experimental challenge with bacterial pathogens. Dis. Aquat. Org. 79, 95–105.
Goncalves, P., Anderson, K., Thompson, E.L., Melwani, A., Parker, L.M., Ross, P.M., Raftos, D.A., 2016. Rapid transcriptional acclimation following transgenerational exposure of oysters to ocean acidification. Mol. Ecol. 25, 4836–4849.
Goncalves, P., Thompson, E.L., Raftos, D.A., 2017. Contrasting impacts of ocean acidification and warming on the molecular responses of CO2-resilient oysters. BMC Genomics 18, 431.
Hille, F., Charpentier, E., 2016. CRISPR-Cas: biology, mechanisms and relevance. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 371.
Humphries, A.T., La Peyre, M.K., Decossas, G.A., 2011. The effect of structural complexity, prey density, and "predator-free space" on prey survivorship at created oyster reef mesocosms. PLoS One 6, e28339.
Huntington, T.G., Balch, W.M., Aiken, G.R., Sheffield, J., Luo, L., Roesler, C.S., Camill, P., 2016. Climate change and dissolved organic carbon export to the Gulf of Maine. J. Geophys. Res. Biogeosci. 121, 2700–2716.
Katherine, E.M., 2013. Fisheries management in a changing climate: lessons from the 2012 ocean heat wave in the Northwest Atlantic. Oceanography 26.
Kleinschuster, S.J., Parent, J., 1995. Sub-clinical infection of oysters (*Crassostrea virginica*) (Gmelin 1791) from Maine by species of the genus *Perkinsus* (Apicomplexa). J. Shellfish Res. 14, 489–491.
La Peyre, J.F., Schafhauser, D.Y., Rizkalla, E.H., Faisal, M., 1995. Production of serine proteases by the oyster pathogen *Perkinsus marinus* (Apicomplexa) *in vitro*. J. Eukaryot. Microbiol. 42, 544–551.
La Peyre, M.K., Casas, S.M., Villalba, A., La Peyre, J.F., 2008. Determination of the effects of temperature on viability, metabolic activity and proliferation of two *Perkinsus* species, and its significance to understanding seasonal cycles of perkinsosis. Parasitology 135, 505–519.
Larsen, P.F., Wilson, K.A., Morse, D., 2013. Observations on the expansion of a relict population of eastern oysters (*Crassostrea virginica*) in a Maine estuary: implications for climate change and restoration. Northeast. Nat. 20, N28–N32.
Lau, Y.T., Gambino, L., Santos, B., Espinosa, E.P., Allam, B., 2018. Transepithelial migration of mucosal hemocytes in *Crassostrea virginica* and potential role in *Perkinsus marinus* pathogenesis. J. Invertebr. Pathol. 153, 122–129.
Lynch, S.A., Armitage, D.V., Coughlan, J., Mulcahy, M.F., Culloty, S.C., 2007. Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian *Bonamia ostreae*. Exp. Parasitol. 115, 359–368.
Madsen, L., Kamp, J., Mellergaard, S., 2013. What can the Limfjord tell us about limiting factors for *Bonamia ostreae* in northern Europe? Bull. Eur. Assoc. Fish Pathol. 33, 165–169.
Maloy, A.P., Barber, B.J., Boettcher, K.J., 2005. A PCR-based diagnostic assay for the detection of *Roseovarius crassostreae* in *Crassostrea virginica* affected by juvenile oyster disease (JOD). Dis. Aquat. Org. 67, 155–162.
Maloy, A.P., Barber, B.J., Boettcher, K.J., 2007a. Use of the 16S-23S rDNA internal transcribed spacer of *Roseovarius crassostreae* for epizootiological studies of juvenile oyster disease (JOD). Dis. Aquat. Org. 76, 151–161.
Maloy, A.P., Ford, S.E., Karney, R.C., Boettcher, K.J., 2007b. *Roseovarius crassostreae*, the etiological agent of juvenile oyster disease (now to be known as Roseovarius Oyster Disease) in *Crassostrea virginica*. Aquaculture 269, 71–83.
Marquis, N.D., Record, N.R., Fernández Robledo, J.A., 2015. Survey for protozoan parasites in Eastern oysters (*Crassostrea virginica*) from the Gulf of Maine using PCR-based assays. Parasitol. Int. 64, 299–302.
Marx, V., 2015. PCR heads into the field. Nat. Methods 12, 393–397.
McLaughlin, S.M., Faisal, M., 1998. *In vitro* propagation of two *Perkinsus* species from the softshell clam *Mya arenaria*. Parasite 5, 341–348.
Meng, K.C., Oremus, K.L., Gaines, S.D., 2016. New England cod collapse and the climate. PLoS One 11, e0158487.
Messerman, N.A., Bowden, T.J., 2016. Survey of potential reservoir species for the oyster parasite multinucleate sphere X (*Haplosporidium nelsoni*) in and around oyster farms in the Damariscotta River estuary, Maine. J. Shellfish Res. 35, 851–856.
Messerman, N.A., Johndrow, K.E., Bowden, T.J., 2014. Prevalence of the protozoan parasite *Haplosporidium nelsoni* in the Eastern oyster, *Crassostrea virginica*, in the Damariscotta River Estuary, in Maine, USA in 2012. Bull. Eur. Assoc. Fish Pathol. 34, 54–62.
Metzger, M.J., Reinisch, C., Sherry, J., Goff, S.P., 2015. Horizontal transmission of clonal cancer cells causes leukemia in soft-shell clams. Cell 161, 255–263.
Metzger, M.J., Villalba, A., Carballal, M.J., Iglesias, D., Sherry, J., Reinisch, C., Muttray,

A.F., Baldwin, S.A., Goff, S.P., 2016. Widespread transmission of independent cancer lineages within multiple bivalve species. Nature 534, 705–709.
Miles, K., 2016. Coast-To-Coast, Boudoin Magazine. Matthew J. O'Donnell, Boudoin College, pp. 8–13.
Mojica, F.J., Montoliu, L., 2016. On the origin of CRISPR-Cas technology: from prokaryotes to mammals. Trends Microbiol. 24, 811–820.
Morga, B., Renault, T., Faury, N., Chollet, B., Arzul, I., 2011. Cellular and molecular responses of haemocytes from *Ostrea edulis* during *in vitro* infection by the parasite *Bonamia ostreae*. Int. J. Parasitol. 41, 755–764.
Morga, B., Renault, T., Faury, N., Arzul, I., 2012. New insights in flat oyster *Ostrea edulis* resistance against the parasite *Bonamia ostreae*. Fish Shellfish Immunol. 32, 958–968.
Nault, D.M., 2016. Neoplasia in Maine Clams. A recent event or old news. In: Shellfish Biosecurity: Risks, Management, and Best Practices. University of Maine Hutchinson Center, Belfast.
Neckles, H.A., 2015. Loss of eelgrass in Casco Bay, Maine, linked to green crab disturbance. Northeast. Nat. 22, 478–500.
Okamoto, N., McFadden, G.I., 2008. The mother of all parasites. Future Microbiol 3, 391–395.
Ottinger, C.A., Lewis, T.D., Shapiro, D.A., Faisal, M., Kaattari, S.L., 2001. Detection of *Perkinsus marinus* extracellular proteins in tissues of the eastern oyster *Crassostrea virginica*: potential use in diagnostic assays. J. Aquat. Anim. Health 13, 133–141.
Pales Espinosa, E., Corre, E., Allam, B., 2014. Pallial mucus of the oyster *Crassostrea virginica* regulates the expression of putative virulence genes of its pathogen *Perkinsus marinus*. Int. J. Parasitol. 44, 305–317.
Pecher, W.T., Alavi, M.R., Schott, E.J., Fernandez-Robledo, J.A., Roth, L., Berg, S.T., Vasta, G.R., 2008. Assessment of the northern distribution range of selected *Perkinsus* species in eastern oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*) with the use of PCR-based detection assays. J. Parasitol. 94, 410–422.
Perry, K.J., Henry, J.Q., 2015. CRISPR/Cas9-mediated genome modification in the mollusc, *Crepidula fornicata*. Genesis 53, 237–244.
Pershing, A.J., Alexander, M.A., Hernandez, C.M., Kerr, L.A., Le Bris, A., Mills, K.E., Nye, J.A., Record, N.R., Scannell, H.A., Scott, J.D., Sherwood, G.D., Thomas, A.C., 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science 350, 809–812.
Pichot, Y., Comps, M., Tigé, G., Grizel, H., Rabouin, M.A., 1979. Research on *Bonamia ostreae* gen. n., sp. n., a new parasite of the flat oyster *Ostrea edulis* L. Rev. Trav. Inst. Pêch. Marit. 43, 131–140.
Proestou, D.A., Vinyard, B.T., Corbett, R.J., Piesz, J., Allen Jr., S.K., Small, J.M., Li, C., Liu, M., DeBrosse, G., Guo, X., Rawson, P., Gómez-Chiarri, M., 2016. Performance of selectively-bred lines of eastern oyster, *Crassostrea virginica*, across eastern US estuaries. Aquaculture 464, 17–27.
Queiroga, F.R., Marques-Santos, L.F., De Medeiros, I.A., Da Silva, P.M., 2016. Effects of salinity and temperature on *in vitro* cell cycle and proliferation of *Perkinsus marinus* from Brazil. Parasitology 143, 475–487.
Record, N.R., 2017. A people's science. Limnol Oceanogr Bull 26, 36–37.
Record, N.R., O'Brien, J.D., Stamieszkin, K., Runge, J.A., 2016. Omic-style statistical clustering reveals old and new patterns in the Gulf of Maine ecosystem. Can. J. Fish. Aquat. Sc. 1–7.
Reece, K.S., Dungan, C.F., Burreson, E.M., 2008. Molecular epizootiology of *Perkinsus marinus* and *P. chesapeaki* infections among wild oysters and clams in Chesapeake Bay, USA. Dis. Aquat. Org. 82, 237–248.
Rick, T.C., Reeder-Myers, L.A., Hofman, C.A., Breitburg, D., Lockwood, R., Henkes, G., Kellogg, L., Lowery, D., Luckenbach, M.W., Mann, R., Ogburn, M.B., Southworth, M., Wah, J., Wesson, J., Hines, A.H., 2016. Millennial-scale sustainability of the Chesapeake Bay native American oyster fishery. Proc. Natl. Acad. Sci. U. S. A. 113, 6568–6573.
Robledo, J.A.F., Gauthier, J.D., Coss, C.A., Wright, A.C., Vasta, G.R., 1998. Species-specificity and sensitivity of a PCR-based assay for *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica*: a comparison with the fluid thioglycollate assay. J. Parasitol. 84, 1237–1244.
Saba, V.S., Griffies, S.M., Anderson, W.G., Winton, M., Alexander, M.A., Delworth, T.L., Hare, J.A., Harrison, M.J., Rosati, A., Vecchi, G.A., Zhang, R., 2016. Enhanced warming of the Northwest Atlantic Ocean under climate change. J. Geophys. Res. 121, 118–132.
Shinn, A.P., Pratoomyot, J., Bron, J.E., Paladini, G., Brooker, E.E., Brooker, A.J., 2014. Economic costs of protistan and metazoan parasites to global mariculture. Parasitology 142, 196–270.
Singh, R.S., Walia, A.K., Kanwar, J.R., 2016. Protozoa lectins and their role in host-pathogen interactions. Biotechnol. Adv. 34, 1018–1029.
Steneck, R.S., Hughes, T.P., Cinner, J.E., Adger, W.N., Arnold, S.N., Berkes, F., Boudreau, S.A., Brown, K., Folke, C., Gunderson, L., Olsson, P., Scheffer, M., Stephenson, E., Walker, B., Wilson, J., Worm, B., 2011. Creation of a gilded trap by the high economic value of the Maine lobster fishery. Conserv. Biol. 25, 904–912.
Sunila, I., Karolus, J., Lang, E.P., Mroczka, M.E., Volk, J., 2000. Transmission of the haplosporidian parasite MSX *Haplosporidium nelsoni* to the eastern oyster *Crassostrea virginica* in an upweller system. Dis. Aquat. Org. 42, 153–155.
Tasumi, S., Vasta, G.R., 2007. A galectin of unique domain organization from hemocytes of the eastern oyster (*Crassostrea virginica*) is a receptor for the protistan parasite *Perkinsus marinus*. J. Immunol. 179, 3086–3098.
Tonelli, F.M.P., Lacerda, S., Tonelli, F.C.P., Costa, G.M.J., de Franca, L.R., Resende, R.R., 2017. Progress and biotechnological prospects in fish transgenesis. Biotechnol. Adv. 35, 832–844.
Vasta, G.R., Feng, C., Bianchet, M.A., Bachvaroff, T.R., Tasumi, S., 2015. Structural, functional, and evolutionary aspects of galectins in aquatic mollusks: from a sweet tooth to the Trojan Horse. Fish Shellfish Immunol. 46, 94–106.
von Itzstein, M., Plebanski, M., Cooke, B.M., Coppel, R.L., 2008. Hot, sweet and sticky: the glycobiology of plasmodium falciparum. Trends Parasitol. 24, 210–218.
Walker, C.W., Bottger, S.A., 2008. A naturally occurring cancer with molecular connectivity to human diseases. Cell Cycle 7, 2286–2289.
Walker, C., Bottger, S.A., Mulkern, J., Jerszyk, E., Litvaitis, M., Lesser, M., 2009. Mass culture and characterization of tumor cells from a naturally occurring invertebrate cancer model: applications for human and animal disease and environmental health. Biol. Bull. 216, 23–39.
Wang, Q., Cao, R., Ning, X., You, L., Mu, C., Wang, C., Wei, L., Cong, M., Wu, H., Zhao, J., 2016. Effects of ocean acidification on immune responses of the Pacific oyster Crassostrea Gigas. Fish & shellfish immunology 49, 24–33.
Wang, Y., Yoshinaga, T., Itoh, N., 2018. New insights into the entrance of *Perkinsus olseni* in the Manila clam, (*Ruditapes philippinarum*). J Invertebr Pathol. 153, 117–121.
Ward, J.R., Lafferty, K.D., 2004. The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? PLoS Biol. 2, E120.
Ye, D., Zhu, Z., Sun, Y., 2015. Fish genome manipulation and directional breeding. Sci. China Life Sci. 58, 170–177.
Zabaleta, A.I., Barber, B.J., 1996. Prevalence, intensity, and detection of *Bonamia ostreae* in *Ostrea edulis* L. in the damariscotta river area, Maine. J. Shellfish Res. 15, 395–400.
Zhong, Z., Niu, P., Wang, M., Huang, G., Xu, S., Sun, Y., Xu, X., Hou, Y., Sun, X., Yan, Y., Wang, H., 2016. Targeted disruption of sp7 and myostatin with CRISPR-Cas9 results in severe bone defects and more muscular cells in common carp. Sci. Rep. 6, 22953.